MONSANTO

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MONSANTO COMPANY
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April 4, 2000

Document Processing Desk (APPL)
Office of Pesticide Programs (7504C)
U.S. Environmental Protection Agency
Room 226A, Crystal Mall #2
1921 Jefferson Davis Highway
Arlington, VA 22202

Attn.: Dr. Janet Andersen, Director, Biopesticides and Pollution Prevention Division

Subject: Request for the Registration of the Plant-Incorporated Protectant, *Bacillus thuringiensis* Cry2Ab Insect Control Protein, as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

Dear Dr. Andersen:

Following US regulatory approvals, Monsanto commercialized Bollgard® cotton (524-478) and YieldGard® corn (524-489) in 1996 and 1997, respectively. These two plant protectant products produce the Cry1Ac and Cry1Ab proteins, respectively and have been adopted broadly by growers because they provide effective protection from the feeding of lepidopteran insect pests. YieldGard corn provides control of the European corn borers and corn earworms, while Bollgard cotton provides control of tobacco budworms, pink bollworms and cotton bollworms. Growers typically apply significantly less insecticide to control these pests, realize higher yields, and achieve greater profitability using these improved varieties, as compared to conventional products.

Gianessi and Carpenter estimated that in 1998 two million acres of corn were treated with insecticides to control the European corn borer, compared to 3.5 million acres in 1995, the year prior to the introduction of the first *B.t.* corn hybrids. This difference represents a reduction of approximately 600,000 pounds of insecticide active ingredient. In cotton, the same authors estimated that the planting of Bollgard varieties reduced insecticide applications by 2 million pounds of insecticides in 1998 alone, compared to the last year prior to the introduction of Bollgard, 1995. For these and other reasons, more than 4.1 million acres of Bollgard and 18 million acres of YieldGard were planted in 1999, or more than 31% of the total cotton and 12% of the total corn acreage, respectively.

Monsanto Company is now requesting the registration of the *Bacillus thuringiensis* subspecies *kurstaki* (*B.t.k.*) Cry2Ab protein and the genetic material necessary for its production in all cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) plants. These new biotech cotton and corn plants were developed using particle acceleration plant transformation procedures to insert the *cry2Ab* insect control gene. These new products provide increased control of insect pests such as the European corn borer, corn earworm, tobacco budworm, pink bollworm and cotton bollworm, as well as armyworm. Combining the Cry2Ab protein with the Cry1Ac and Cry1Ab proteins already in the marketplace, or using the Cry2Ab protein as a replacement for the current products, will provide an additional tool to delay the development of lepidopteran resistance in cotton and corn. These products, in combination with a refuge and the other components of Monsanto's resistance management plan, represent a substantial program to significantly delay the development of insect resistance to cotton or corn containing the Cry1Ac or Cry1Ab protein, respectively.

Under parts (C) and (D) of section 3 (c) (5) of FIFRA, a pesticide must perform its intended function without unreasonable adverse effects on the environment when used in accordance with widespread and commonly recognized practice. FIFRA defines unreasonable adverse effects to include both risks to human health and ecological effects. We respectfully conclude that the data and information on Cry2Ab contained in this submission, developed according to EPA guidelines, fully support the EPA determinations necessary for registration of this protein under this section. An exemption from the requirement of a tolerance for this protein on all raw agricultural commodities has been requested under a separate submission to the Agency through an amendment to Tolerance Petition PP 7F4888 (Docket Number PF-768). All data and information demonstrating the human health safety of this protein were provided in that request.

Monsanto desires to commercialize these products in the 2002 production season and therefore, requests that the scientific review and registration of these products be concluded by September 1, 2001. Should you have any questions regarding this request, please contact Karen Gustafson at 636-737-6870, Terry Stone at 636-737-6547 or Russell Schneider at 202-383-2866.

Sincerely,

Karen S. Gust

Karen Gustafson
Regulatory Affairs Manager
Cotton

Terry Stone
Regulatory Affairs Manager
Corn

cc: 99-858E
Russ Schneider

TRANSMITTAL DOCUMENT

SUBMITTED BY

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

REGULATORY ACTION IN SUPPORT OF WHICH THIS PACKAGE IS SUBMITTED

Request for the Registration of the Plant-Expressed Protectant,
Bacillus thuringiensis Cry2Ab Insect Control Protein,
as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

TRANSMITTAL DATE

April 4, 2000

LIST OF SUBMITTED DOCUMENTS

ADMINISTRATIVE MATERIALS

Volume 1: Administrative Materials in Support of the Request for the Registration of the Plant-Incorporated Protectant, *Bacillus thuringiensis* Cry2Ab Insect Control Protein, as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

MRID Number ______________________

PRODUCT CHARACTERIZATION

Volume 2: K.A. Hamilton and A. Reed, Field Report: Production of Tissue Samples from Insect Protected Cotton Events Grown in 1998 U.S. Field Trials, Monsanto Report Number MSL-16019, an unpublished study conducted by Monsanto Company.

MRID Number 45086301

Volume 3: J.D. Colyer, C.C. Deatherage and K.C. Glenn, Production of Tissue Samples from Corn Events MON 840, MON 841 and MON 843 in the 1998 U.S. Field Trials, Monsanto Report Number MSL-16041, an unpublished study conducted by Monsanto Company.

MRID Number 45086302

Volume 4: S.C. Doherty, K.A. Hamilton, R.P. Lirette and I. Borovkova, Amended Report for Molecular Characterization of Cotton Event 15985, Monsanto Report Number MSL-16620, an unpublished study conducted by Monsanto Company.

MRID Number 45086303

Volume 5: J.C. Jennings, I.G. Borovkova, R.P. Lirette, Molecular Analysis of Corn Event 840, Monsanto Report Number MSL-16080, an unpublished study conducted by Monsanto Company.

MRID Number 45086304

Volume 6: G. Holleschak, R.S. Thoma, T.C. Lee, R.E. Hileman and J.D. Astwood, Assessment of the Equivalence of Proteins Expressed in Corn Event MON 840, Monsanto Report Number MSL-16224, an unpublished study conducted by Monsanto Company.

MRID Number 45086305

Volume 7: B.P. Tonnu, P.R. Sanders, J.C. Jennings, Insect Protection Protein 2 and NPTII Protein Levels in Samples Collected from Corn Event MON 840 in the 1998 U.S. Field Trials, Monsanto Report Number MSL-15708, an unpublished study conducted by Monsanto Company.

MRID Number 45086306

ENVIRONMENTAL FATE AND ECOLOGICAL EFFECTS

Volume 8: V.L. Maggi, Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Honey Bee Larvae, Monsanto Report Number MSL-16175, an unpublished study conducted by California Agricultural Research, Inc.

MRID Number 45086307

Volume 9: V.L. Maggi, Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis melifera* L.). Monsanto Report Number MSL-16176, an unpublished study conducted by California Agricultural Research, Inc.

MRID Number 45086308

Volume 10: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*), Monsanto Report Number MSL-16171, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086309

Volume 11: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*), Monsanto Report Number MSL-16173, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086310

Volume 12: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*), Monsanto Report Number MSL-16172, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086311

Volume 13: K.R. Drottar and H.O. Krueger, Insect Protection Protein 2 in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Monsanto Report Number MSL-16180, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086312

Volume 14: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate, Monsanto Report Number MSL-16177, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086313

Volume 15: D. Teixeira, Assessment of Chronic Toxicity of Cotton Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*), Monsanto Report Number MSL-16174, an unpublished study conducted by Springborn Laboratories, Inc.

MRID Number 45086314

Volume 16: D. Teixeira, Assessment of Chronic Toxicity of Corn Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*), Monsanto Report Number MSL-16181, an unpublished study conducted by Springborn Laboratories, Inc.

MRID Number 45086315

Volume 17: S.P. Gallagher, J. Grimes and J.B. Beavers, Insect Protection Protein 2 in Cotton Seed Meal: A Dietary Toxicity Study with the Northern Bobwhite, Monsanto Report Number MSL-16178, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086316

Volume 18: S.P. Gallagher, J. Grimes and J.B. Beavers, Insect Protection Protein 2 in Corn Grain: A Dietary Toxicity Study with the Northern Bobwhite, Monsanto Report Number MSL-16182, an unpublished study conducted by Wildlife International, Ltd.

MRID Number 45086317

Volume 19: M.H. Li and E.H. Robinson, Evaluation of Cottonseed Meal Derived From Insect Protected Cotton Lines 15813 as a Feed Ingredient for Catfish, Monsanto Report Number MSL-16179, an unpublished study conducted by the Thad Cochran National Warmwater Aquaculture Center.

MRID Number 45086318

Volume 20: M.H. Li and E.H. Robinson, Evaluation of Corn Lines MON 840 and MON 841 as a Feed Ingredient for Catfish, Monsanto Report Number MSL-16183, an unpublished study conducted by the Thad Cochran National Warmwater Aquaculture Center.

MRID Number 45086319

COMPANY OFFICIAL Karen S. Gust 4/4/00
Karen S. Gustafson Date
Regulatory Affairs Manager

COMPANY OFFICIAL Terry B. Stone 4/4/00
Terry B. Stone Date
Regulatory Affairs Manager

COMPANY NAME: MONSANTO COMPANY

COMPANY CONTACT: Dr. Russell Schneider (202) 383-2866

Summary Title

Administrative Materials in Support of the Request for the Registration of the Plant-Incorporated Protectant, *Bacillus thuringiensis* Cry2Ab Insect Control Protein, as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

Data Requirement

Administrative Materials Required for Registration

Authors

Karen S. Gustafson
Terry B. Stone
Bruce G. Hammond
James D. Astwood
Michael J. McKee
Graham Head

Registrant Submitting Date

April 4, 2000

Registrant Submitting

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

99-858E

Volume 1 of 20

STATEMENT OF NO CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A),(B), or (C).

> "We submitted this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material by EPA according to FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to EPA."

COMPANY: MONSANTO COMPANY

COMPANY AGENT: Karen S. Gust
Karen S. Gustafson
Regulatory Affairs Manager

COMPANY AGENT:
Terry B. Stone
Regulatory Affairs Manager

DATE: April 4, 2000

GLP COMPLIANCE STATEMENT

The enclosed data, presented to the Environmental Protection Agency in support of the request for the registration of the plant-incorporated protectant, *Bacillus thuringiensis* Cry2Ab2 insect control protein, have been summarized from studies which were conducted in compliance with the Good Laboratory Practice Standards, 40 CFR 160, as set down in Federal Register, 54, 34052-74, 17 August 1989.

Karen S. Gust 4/4/00

Karen S. Gustafson Date
Regulatory Affairs Manager
Sponsor/Submitter

4/4/00

Terry B. Stone Date
Regulatory Affairs Manager
Sponsor/Submitter

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Volume 9: V.L. Maggi, Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis melifera* L.), Monsanto Report Number MSL-16176.

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Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*), Monsanto Report Number MSL-16171.

Volume 11: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*), Monsanto Report Number MSL-16173.

Volume 12: S.J. Palmer and H.O. Krueger, Insect Protection Protein 2: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*), Monsanto Report Number MSL-16172.

Volume 13: K.R. Drottar and H.O. Krueger, Insect Protection Protein 2 in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*), Monsanto Report Number MSL-16180.

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Volume 19: M.H. Li and E.H. Robinson, Evaluation of Cottonseed Meal Derived From Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish, Monsanto Report Number MSL-16179.

Volume 20: M.H. Li and E.H. Robinson, Evaluation of Insect Protected Corn Lines MON 840 and MON 841 as a Feed Ingredient for Catfish, Monsanto Report Number MSL-16183.

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NOTE TO REVIEWER

Many of the studies contained within this submission identify various corn and cotton events that have been given specific designations. Bollgard II cotton events are designated 15985 and 15813; the new corn event is designated as MON 840. Each of these specific events contains the *cry2Ab* gene and expresses the Cry2Ab protein. The Cry2Ab protein expressed in corn differs by only one amino acid from that expressed in cotton and thus these proteins are 99.8% sequence identical. The designation Cry2Ab2 is also used in the technical reports to describe the Cry2Ab protein. This designation has been used for tracking purposes within Monsanto, as the revised nomenclature system currently applies only to wild-type sequences (Crickmore, *et al.*, 1998).

The Cry2Ab proteins expressed by these cotton and corn events are 99.8% sequence identical to the Cry2Ab protein encoded by the genetic material in the genome of *Bacillus thuringiensis* subsp. *kurstaki*. In both cases, a single amino acid (the second one in the sequence) has been altered, although the changes for corn and cotton differ. The Cry2Ab protein expressed in transgenic plants contains a single amino acid addition at the N-terminus compared to the wild-type amino acid sequence, due to the creation of a restriction enzyme cleavage site introduced for cloning purposes. Thus both proteins fall well within the >95% amino acid sequence identity required for proteins within the Cry2Ab class as described by Crickmore, *et al.*, 1998.

Data supporting the human and animal safety assessment of the Cry2Ab protein has been previously submitted as a part of the request for tolerance exemption and is therefore referenced in this volume (MRIDs 44999301, 44966601-5).

ABBREVIATIONS

B.t.	*Bacillus thuringiensis*
B.t.k.	*Bacillus thuringiensis* subsp. *kurstaki*
Cry1Ac	Naturally occurring insecticidal protein produced by *B.t.k.* and expressed in Bollgard® cotton and Bollgard® II cotton products
Cry1Ab	Naturally occurring insecticidal protein produced by *B.t.k.* and expressed in YieldGard® corn and YieldGard® II corn products
Cry2Aa	Naturally occurring insecticidal protein produced by *B.t.k.* and present in commercial microbial *B.t.* formulations
Cry2Ab	Insecticidal protein expressed in Bollgard II cotton event 15985 and corn event MON 840
IPP2	Insecticidal protein derived from *B.t.k.*, also known as CryIIB, CryB2, and CryIIAb
IPM	Integrated Pest Management
IRM	Insect Resistance Management
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
SAP	Scientific Advisory Panel
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid

Section I. Administrative Materials

Please read instructions on reverse before completing form. Form Approved. OMB No. 2070-0060. Approval expires 2-28-95

EPA United States **Environmental Protection Agency** Washington, DC 20460

[✓] **Registration**
[ ] **Amendment**
[ ] **Other**

OPP Identifier Number: 270766 ~~258033~~

Application for Pesticide - Section I

1. Company/Product Number Monsanto/ 524 - LEE	2. EPA Product Manager Phil Hutton	3. Proposed Classification [✓] None [ ] Restricted
4. Company/Product (Name) Monsanto / Crv2Ab Protein	PM# 90	

5. Name and Address of Applicant *(Include ZIP Code)*
Monsanto Company
600 13th Street, Suite 660
Washington, DC 20005

[ ] *Check if this is a new address*

6. Expedited Reveiw. In accordance with FIFRA Section 3(c)(3)(b)(i), my product is similar or identical in composition and labeling to:
EPA Reg. No. 524 -
Product Name Cry2Ab Protein

Section - II

[ ] Amendment - Explain below.
[ ] Resubmission in response to Agency letter dated ________
[ ] Notification - Explain below.
[ ] Final printed labels in repsonse to Agency letter dated ________
[ ] "Me Too" Application.
[✓] Other - Explain below.

Explanation: Use additional page(s) if necessary. (For section I and Section II.)
Request for registration of the plant-incorporated protectant, Bacillus thuringiensis Cry2Ab Insect Control Protein as produced in corn and cotton

Section - III

1. Material This Product Will Be Packaged In:

Child-Resistant Packaging	Unit Packaging		Water Soluble Packaging		2. Type of Container
[ ] Yes [✓] No	[ ] Yes [✓] No		[ ] Yes [✓] No		[ ] Metal [ ] Plastic [ ] Glass [ ] Paper [✓] Other (Specify) Plant Cells
Certification must be submitted	If "Yes" Unit Packaging wgt.	No. per container	If "Yes" Package wgt	No. per container	

3. Location of Net Contents Information [ ] Label [ ] Container	4. Size(s) Retail Container	5. Location of Label Directions

6. Manner in Which Label is Affixed to Product: [ ] Lithograph Paper glued Stenciled [✓] Other ________

Section - IV

1. Contact Point *(Complete items directly below for identification of individual to be contacted, if necessary, to process this application.)*

Name	Title	Telephone No. (Include Area Code)
Russell P. Schneider	Regulatory Director	(202) 383 - 2866

Certification

I certify that the statements I have made on this form and all attachments thereto are true, accurate and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature Karen S. Gust	3. Title Regulatory Affairs Manager	6. Date Application Received (Stamped)
4. Typed Name Karen S. Gustafson	5. Date April 4, 2000	

MONSANTO 99-858E Page 12 of 70

Pages 21-24 Confidential Statement of Formula may be entitled to confidential treatment

Form Approved OMB No. 2070-0060

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Paperwork Reduction Act Notice: The public reporting burden for this collection of information is estimated to average 1.25 hours per response for registration and 0.25 hours per response for reregistration and special review activities, including time for reading the instructions and completing the necessary forms. Send comments regarding burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden to: Director, OPPE Information Management Division (2137), U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460.
Do not send the completed form to this address.

Certification with Respect to Citation of Data

Applicant's/Registrant's Name, Address, and Telephone Number Karen S. Gustafson, Monsanto Company, 600 13th St, Suite 660, Washington, DC 20005	EPA Registration Number/File Symbol 524 -
Active Ingredient(s) and/or representative test compound(s) Cry2Ab Protein	Date April 4, 2000
General Use Pattern(s) (list all those claimed for this product using 40 CFR Part 158)	Product Name Cry2Ab Protein

NOTE: If your product is a 100% repackaging of another purchased EPA-registered product labeled for all the same uses on your label, you do not need to submit this form. You must submit the Formulator's Exemption Statement (EPA Form 8570-27).

☐ I am responding to a Data-Call-In Notice, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

SECTION I: METHOD OF DATA SUPPORT (Check one method only)

☐ I am using the cite-all method of support, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

☑ I am using the selective method of support (or cite-all option under the selective method), and have included with this form a completed list of data requirements (the Data Matrix form must be used).

SECTION II: GENERAL OFFER TO PAY

[Required if using the cite-all method or when using the cite-all option under the selective method to satisfy one or more data requirements]

☐ I hereby offer and agree to pay compensation, to other persons, with regard to the approval of this application, to the extent required by FIFRA.

SECTION III: CERTIFICATION

I certify that this application for registration, this form for reregistration, or this Data-Call-In response is supported by all data submitted or cited in the application for registration, the form for reregistration, or the Data-Call-In response. In addition, if the cite-all option or cite-all option under the selective method is indicated in Section I, this application is supported by all data in the Agency's files that (1) concern the properties or effects of this product or an identical or substantially similar product, or one or more of the ingredients in this product; and (2) is a type of data that would be required to be submitted under the data requirements in effect on the date of approval of this application if the application sought the initial registration of a product of identical or similar composition and uses .

✓ I certify that for each exclusive use study cited in support of this registration or reregistration, that I am the original data submitter or that I have obtained the written permission of the original data submitter to cite that study.

I certify that for each study cited in support of this registration or reregistration that is not an exclusive use study, either: (a) I am the original data submitter; (b) I have obtained the permission of the original data submitter to use the study in support of this application; (c) all periods of eligibility for compensation have expired for the study; (d) the study is in the public literature; or (e) I have notified in writing the company that submitted the study and have offered (I) to pay compensation to the extent required by sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA; and (ii) to commence negotiations to determine the amount and terms of compensation, if any, to be paid for the use of the study.

I certify that in all instances where an offer of compensation is required, copies of all offers to pay compensation and evidence of their delivery in accordance with sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA are available and will be submitted to the Agency upon request. Should I fail to produce such evidence to the Agency upon request, I understand that the Agency may initiate action to deny, cancel or suspend the registration of my product in conformity with FIFRA.

I certify that the statements I have made on this form and all attachments to it are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature Karen S. Gust	Date April 4, 2000	Typed or Printed Name and Title Karen S. Gustafson, Regulatory Affairs Manager

Form Approved OMB No. 2070-0060

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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Date April 4, 2000	EPA Reg No./File Symbol 524-	Page 1 of 6
Applicant's/Registrant's Name & Address Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198	Product Cry2Ab	

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Vol. 1. Administrative Materials in Support of the Request for the Registration of the Plant-Incorporated Protectant, *Bacillus thuringiensis* Cry2Ab Insect Control Protein, as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)		Monsanto Company	OWN	This submission
	Vol. 2. Field Report: Production of Tissue Samples from Insect Protected Cotton Events Grown in 1998 U.S. Field Trials		Monsanto Company	OWN	This submission
	Vol. 3. Production of Tissue Samples from Corn Events MON 840, MON 841 and MON 843 in the 1998 U.S. Field Trials		Monsanto Company	OWN	This submission
	Vol. 4. Molecular Characterization of Cotton Event 15985		Monsanto Company	OWN	This submission
	Vol. 5. Molecular Analysis of Corn Event 840		Monsanto Company	OWN	This submission
	Vol. 6. Assessment of the Equivalence of Proteins Expressed in Corn Event MON 840		Monsanto Company	OWN	This submission
	Vol. 7. Insect Protection Protein 2 and NPTII Protein Levels in Samples Collected from Corn Event MON 840 in the 1998 U.S. Field Trials		Monsanto Company	OWN	This submission
	Vol. 8. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Honey Bee Larvae (*Apis melifera* L.)		Monsanto Company	OWN	This submission
	Vol. 9. Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis melifera* L.)		Monsanto Company	OWN	This submission

Signature	Name and Title	Date
Karen S. Gust	Karen S. Gustafson, Regulatory Affairs Manager	4/4/00

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Applicant's/Registrant's Name & Address
Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198

Product
Cry2Ab

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Vol. 10. Insect Protection Protein 2: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*)		Monsanto Company	OWN	This submission
	Vol. 11. Insect Protection Protein 2: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*)		Monsanto Company	OWN	This submission
	Vol. 12. Insect Protection Protein 2: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*)		Monsanto Company	OWN	This submission
	Vol. 13. Insect Protection Protein 2 in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)		Monsanto Company	OWN	This submission
	Vol. 14. Insect Protection Protein 2 in Corn Pollen: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate		Monsanto Company	OWN	This submission
	Vol. 15. Assessment of Chronic Toxicity of Cotton Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*)		Monsanto Company	OWN	This submission
	Vol. 16. Assessment of Chronic Toxicity of Corn Tissue Containing Insect Protection Protein 2 to Collembola (*Folsomia candida*)		Monsanto Company	OWN	This submission
	Vol. 17. Insect Protection Protein 2 in Cotton Seed Meal: A Dietary Toxicity Study with the Northern Bobwhite		Monsanto Company	OWN	This submission

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Applicant's/Registrant's Name & Address: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198

Product: Cry2Ab

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Vol. 18. Insect Protection Protein 2 in Corn Grain: A Dietary Toxicity Study with the Northern Bobwhite		Monsanto Company	OWN	This submission
	Vol. 19. Evaluation of Cottonseed Meal Derived from Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish		Monsanto Company	OWN	This submission
	Vol. 20. Evaluation of Insect Protected Corn Lines MON 840 and MON 841 as a Feed Ingredient for Catfish		Monsanto Company	OWN	This submission
	Vol. 1. Admin. Materials in Support of the Amendment to the Request for Exemption from the Requirement of a Tolerance (Petition PP 7F4888)	44966600	Monsanto Company	OWN	
	Vol. 2. Characterization of Insect Protection Protein 2 (IPP2) Produced by Fermentation	44999301	Monsanto Company	OWN	
	Vol. 3. Protein Levels in Insect Protected Cotton Samples Produced in the 1998 U.S. Field Trials	44966601	Monsanto Company	OWN	
	Vol. 4. Acute Oral Toxicity Study of Insect Protection Protein 2 (IPP2) in Mice	44966602	Monsanto Company	OWN	
	Vol. 5. Assessment of the *in vitro* Digestibility of Insect Protection Protein 2 (IPP2) Utilizing Mammalian Digestive Fate Models	44966603	Monsanto Company	OWN	
	Vol. 6. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing an Allergen Database	44966604	Monsanto Company	OWN	

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Applicant's/Registrant's Name & Address

Karen S. Gustafson / Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198

Product

Cry2Ab

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Vol. 7. Bioinformatics Analysis of Insect Protection Protein 2 (IPP2) Sequence Utilizing Toxin and Public Domain Genetic Databases	44966605	Monsanto Company	OWN	
	Bacillus thuringiensis subsp. *kurstaki* P2A Insecticidal Protein (CryIIA Protein) Shares no Significant Sequence Similarity with Proteins Associated with Allergy or Coelaic Disease	44235304	Monsanto Company	OWN	
	Bacillus thuringiensis subsp. *kurstaki* P2A Insecticidal Protein (CryIIA Protein) is Homologous to Proteins of the *Bacillus thuringiensis* Insecticidal Crystal Protein Gene Family, but not to Protein Toxins Found in Public Domain Sequence Databases	44235305	Monsanto Company	OWN	
	Assessment of the Digestibility of Purified *B.t.k.* P2 Protein *in vitro* Using Mammalian Digestive Fate Models	44235306	Monsanto Company	OWN	
	Preparation and Confirmation of Doses for an Acute Oral Toxicity Study with *Bacillus thuringiensis* var. *kurstaki* Strain HD-1 (CryIIA) Protein in Albino Mice	44235307	Monsanto Company	OWN	
	Acute Oral Toxicity Study of *Bacillus thuringiensis* var. *kurstaki* strain HD-1 CryIIA (*B.t.k.* P2) Protein in Albino Mice	44310303	Monsanto Company	OWN	
	Administrative Materials in Support of the Request for the Registration and Exemption from the Requirement of a Tolerance for the Plant Pesticide *Bacillus thuringiensis* var. *kurstaki* (B.t.k.) Insect Control Protein (CryIIA)		Monsanto Company	OWN	
	1994 Evaluation of Insect Resistant Cotton Lines in U.S. Field Test Locations	44310301	Monsanto Company	OWN	

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Product

Cry2Ab

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Field Summary and Analytical Evaluation of Insect Resistant Cotton Lines in 1995 U.S. Field Trial Locations	44310302	Monsanto Company	OWN	
	Molecular Characterization of Insect Resistant Cotton Lines 1849, 1983, and 2020	44235301	Monsanto Company	OWN	
	Characterization of *Bacillus thuringiensis* var. *kurstaki* strain HD-1 [CryIIA] (B.t.k. P2) Protein Produced by Large-Scale Fermentation of Genetically Modified *Echerichia coli*	44235302	Monsanto Company	OWN	
	Assessment of the Equivalence of *Bacillus thuringiensis* var. *kurstaki* strain HD-1 (CryIIA) (abbreviated as B.t.k. P2) Protein Produced in *Echerichia coli* and Insect Resistant Cotton	44235303	Monsanto Company	OWN	
	Soil Degradation of *Bacillus thuringiensis* subsp. *kurstaki* CryIIA Insecticideal Protein in Cotton Tissue: Comparison of Laboratory Microcosm and Field Studies	44235308	Monsanto Company	OWN	
	Host Activity Spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* Protein: Effects on Lepidoptera, Diptera, and Non-target Arthropods	44235309	Monsanto Company	OWN	
	Evaluation of the Dietary Effect(s) of Purified *Bacillus thuringiensis* subsp. *kurstaki* HD-1 P2 Protein on Honey Bee Larvae	44310304	Monsanto Company	OWN	
	Analysis of *Bacillus thuringiensis* var. *kurstaki* HD-1 P2 (B.t.k. P2 Protein) Concentration in the Test Diet Used for the Larval Honey Bee Study	44235310	Monsanto Company	OWN	
	Evaluation of the Dietary Effect(s) of Purified *Bacillus thuringiensis* subsp. *kurstaki* HD-1 P2 Protein on Adult Honey Bee	44310305	Monsanto Company	OWN	

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Product

Cry2Ab

Ingredient Cry2Ab Protein and the genetic material necessary for its production in cotton and corn

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Analysis of *Bacillus thuringiensis* var. *kurstaki* HD-1 P2 (B.t.k. P2 Protein) Concentration in the Test Diet Used for the Adult Honey Bee Study	44235311	Monsanto Company	OWN	
	Bacillus thuringiensis subsp. *kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*)	44235312	Monsanto Company	OWN	
	Bacillus thuringiensis subsp. *kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*)	44235313	Monsanto Company	OWN	
	Bacillus thuringiensis subsp. *kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*)	44235314	Monsanto Company	OWN	
	Effect of the *Bacillus thuringiensis* Insecticidal Proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Colembola)	44235315	Monsanto Company	OWN	
	CryII Protein from *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k. P2 Protein): An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate	44235316	Monsanto Company	OWN	
	Resistance Management Strategy for BollgardTM Expressing the CryIIA Protein of *Bacillus thuringiensis* subsp. *kurstaki*	44235317	Monsanto Company	OWN	

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Section II. Summary of Application

Request for the Registration of the Plant-Incorporated Protectant,
***Bacillus thuringiensis* Cry2Ab2 Insect Control Protein,**
as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

A. Introduction

Following EPA, USDA and FDA approvals and registrations, Monsanto commercialized Bollgard® cotton (524-478) and YieldGard® corn (524-489) in 1996 and 1997, respectively (EPA 1995a; EPA 1996a). These two plant protectant products produce the Cry1Ac and Cry1Ab proteins, respectively and have been adopted broadly by growers because they provide effective protection from the feeding of lepidopteran insect pests. YieldGard corn provides control of European corn borers and corn earworms, while Bollgard cotton provides control of tobacco budworms, pink bollworms and cotton bollworms. Growers typically apply significantly less insecticide to control these pests, realize higher yields and achieve greater profitability using these improved varieties, as compared to conventional products.

Gianessi and Carpenter (1999) estimated that in 1998 two million acres of corn were treated with insecticides to control the European corn borer, compared to 3.5 million acres in 1995, the year prior to the introduction of the first *B.t.* corn hybrids. This difference represents a reduction of approximately 600,000 pounds of insecticide active ingredient (Gianessi and Carpenter, 1999). In cotton, the same authors estimated that the planting of Bollgard cotton varieties reduced insecticide applications by two million pounds of insecticides in 1998 alone, compared to the last year prior to the introduction of Bollgard, 1995. For these and other reasons, more than 4.1 million acres of Bollgard and 18 million acres of YieldGard varieties were planted in 1999, or more than 31% of the total cotton and 12% of the total corn acreage, respectively (USDA, 1999a).

Monsanto Company is now requesting the registration of the *Bacillus thuringiensis* subspecies *kurstaki* (*B.t.k.*) Cry2Ab protein and the genetic material necessary for its production in all cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.) plants. These new biotech cotton and corn plants were developed using particle acceleration plant transformation procedures to insert the *cry2Ab* insect control gene. These new products provide increased control of insect pests such as the European corn borer, corn earworm, tobacco budworm and pink bollworm, as well as armyworm. Combining the Cry2Ab protein with the Cry1Ac and Cry1Ab proteins already in the marketplace, or using the Cry2Ab protein as a replacement for the current products, will provide an additional tool to delay the development of lepidopteran resistance in cotton and corn. These products, in combination with a refuge and the other components of Monsanto's resistance management plan, represent a substantial program to significantly delay the development of insect resistance to cotton or corn containing the Cry1Ac or Cry1Ab protein, respectively.[1]

Under parts (C) and (D) of section 3 (c) (5) of FIFRA, a pesticide must perform its intended function without unreasonable adverse effects on the environment when used in accordance with widespread and commonly recognized practices. FIFRA defines

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[1] Bollgard® and YieldGard® are registered trademarks of Monsanto Company

unreasonable adverse effects to include both risks to human health and ecological effects. We respectfully conclude that the data and information on Cry2Ab contained in this submission, developed according to EPA guidelines, fully support the EPA determinations necessary for registration of this protein under this section. An exemption from the requirement of a tolerance for this protein on all raw agricultural commodities has been requested under a separate submission to the Agency through an amendment to Tolerance Petition PP 7F4888 (Docket Number PF-768). All data and information demonstrating the human health safety of this protein were provided in that request. The following summary summarizes the data and evidence, which establish the human and environmental safety of the Cry2Ab protein.

B. Nomenclature
Cry2Ab is a protein derived from *Bacillus thuringiensis* and has also been designated Cry2Ab2, CryIIB, CryB2 or CryIIAb (Liang and Dean, 1994; Widner and Whiteley, 1990; Crickmore, *et al.*, 1998) or Insect Protection Protein 2 (IPP2). In the current nomenclature scheme for Cry proteins, names are assigned according to amino acid similarity to established holotype proteins as defined by Crickmore, *et al.* (1998). In this nomenclature, Cry proteins with similar amino acid sequences are grouped together. Cry proteins with the same Arabic numeral, *e.g.*, Cry2, share at least a 45% amino acid sequence identity. Those with the Arabic numeral and upper case letter, *e.g.*, Cry2A, share at least a 75% sequence identity. Finally, Cry proteins with the same Arabic numeral, upper case letter and lower case letter, *e.g.*, Cry2Ab, share a greater than 95% sequence identity.

C. *cry2Ab* Gene
Bacillus thuringiensis (*B.t.*) is a gram-positive bacterium commonly present in soil that has been used commercially in the U.S. since 1958 to produce microbial-derived products with insecticidal activity (EPA, 1988). *Bacillus thuringiensis* subsp. *kurstaki*, present in commercial microbial pest control products such as DIPEL® and Crymax®[2], contains both the *cry2Aa* and *cry2Ab* genes. The *cry2Aa* gene is expressed in these commercial products; however, the *cry2Ab* gene is a pseudogene, which even though present is not expressed due to an inefficient *cry2Ab* promoter (Dankocsik *et al.*, 1990). Therefore, the Cry2Ab protein is not naturally expressed in soil bacteria or sprayable microbial formulations (Widner and Whiteley, 1990; Crickmore, *et al.*, 1994). Both the *cry2Aa* and *cry2Ab* genes are located on the same 100 MDa plasmid (Donovan, *et al.*, 1988; 1989) and the sequence of the *cry2Ab* gene has been fully characterized (Widner and Whiteley, 1990).

D. Cry2Ab Protein
Assessment of the safety of the Cry2Ab protein produced in corn and cotton plants required production of sufficient quantities of material to conduct safety tests. Due to the extremely low levels of Cry2Ab protein produced in plants, it was necessary to produce Cry2Ab protein by bacterial fermentation to conduct the safety studies.

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[2] DIPEL and Crymax are registered trademarks of Abbott and Ecogen, respectively.

Since the Cry2Ab gene is not naturally expressed in *Bacillus thuringiensis* subsp. *kurstaki*, as described in the section above, the *cry2Ab* pseudogene with the necessary promoter region was cloned into *Bacillus thuringiensis* strain EG7699. The Cry2Ab protein expression product was then isolated and purified from the EG7699 bacterial strain. The Cry2Ab protein product (GenBank Accession No. X55416) is 633 amino acids in length, with an approximate mass of 71 kDa (Widner and Whiteley, 1990; Dankocsik *et al.*, 1990). The coding region of the Cry2Ab protein introduced into plants is shown in Figure 1. An additional amino acid (position 2, Figure 1) was introduced to create a restriction enzyme cleavage site for cloning purposes. The coding region of the Cry2Ab protein is highly similar to the Cry2Aa protein, sharing 88% amino acid sequence identity (Widner and Whiteley, 1990; Dankocsik *et al.*, 1990) and 97% amino acid similarity (amino acid identities and conservative replacements). The Cry2Ab protein that is produced in transgenic corn and cotton plants is predicted to contain an additional three amino acids due to processing of the chloroplast transit peptide (underlined positions 77-79, Figures 2 and 3).

Figure 1. Amino Acid Sequences of Cry2Ab and Cry2Aa Proteins

```
                10        20        30        40        50        60
Cry2Ab  MDNSVLNSGRTTICDAYNVAAHDPFSFQHKSLDTVQKEWTEWKKNNHSLYLDPIVGTVAS
          |:|||||||||||||||:|||||||:||||||:|||| |||:::||||: |:||||:|
Cry2Aa   MNNVLNSGRTTICDAYNVVAHDPFSFEHKSLDTIQKEWMEWKRTDHSLYVAPVVGTVSS
                 10        20        30        40        50

                70        80        90       100       110       120
        FLLKKVGSLVGKRILSELRNLIFPSGSTNLMQDILRETEKFLNQRLNTDTLARVNAELTG
        |||||||||:|||||||| ::||||||||||||||||||:|||||||||||||||||| |
        FLLKKVGSLIGKRILSELWGIIFPSGSTNLMQDILRETEQFLNQRLNTDTLARVNAELIG
       60        70        80        90       100       110

               130       140       150       160       170       180
        LQANVEEFNRQVDNFLNPNRNAVPLSITSSVNTMQQLFLNRLPQFQMQGYQLLLLPLFAQ
        ||||::|||:||||||||::| ||||||||||||||||||||||||:|||||||||||||
        LQANIREFNQQVDNFLNPTQNPVPLSITSSVNTMQQLFLNRLPQFQIQGYQLLLLPLFAQ
      120       130       140       150       160       170

               190       200       210       220       230       240
        AANLHLSFIRDVILNADEWGISAATLRTYRDYLKNYTRDYSNYCINTYQSAFKGLNTRLH
        |||:|||||||||||||||||||||||||||||:|||||||||||||||:||:|||||||
        AANMHLSFIRDVILNADEWGISAATLRTYRDYLRNYTRDYSNYCINTYQTAFRGLNTRLH
      180       190       200       210       220       230

               250       260       270       280       290       300
        DMLEFRTYMFLNVFEYVSIWSLFKYQSLLVSSGANLYASGSGPQQTQSFTSQDWPFLYSL
        ||||||||||||||||||||||||||||:|||||||||||||||||||||:|:|||||||
        DMLEFRTYMFLNVFEYVSIWSLFKYQSLMVSSGANLYASGSGPQQTQSFTAQNWPFLYSL
      240       250       260       270       280       290

               310       320       330       340       350       360
        FQVNSNYVLNGFSGARLSNTFPNIVGLPGSTTTHALLAARVNYSGGISSGDIGASPFNQN
        |||||||:|:|:||:||| ||||| |||||||||:| :||||||||:||| |||: :|:|
        FQVNSNYILSGISGTRLSITFPNIGGLPGSTTTHSLNSARVNYSGGVSSGLIGATNLNHN
      300       310       320       330       340       350

               370       380       390       400       410       420
        FNCSTFLPPLLTPFVRSWLDSGSDREGVATVTNWQTESFETTLGLRSGAFTARGNSNYFP
        ||||| |||| |||||||||||:||||||| ||||||||:|||:|| |||:|||||||||
        FNCSTVLPPLSTPFVRSWLDSGTDREGVATSTNWQTESFQTTLSLRCGAFSARGNSNYFP
      360       370       380       390       400       410

               430       440       450       460       470       480
        DYFIRNISGVPLVVRNEDLRRPLHYNEIRNIASPSGTPGGARAYMVSVHNRKNNIHAVHE
        |||||||||||||:||||| ||||||:|||| ||||||||||||:||||||||||:|::|
        DYFIRNISGVPLVIRNEDLTRPLHYNQIRNIESPSGTPGGARAYLVSVHNRKNNIYAANE
      420       430       440       450       460       470

               490       500       510       520       530       540
        NGSMIHLAPNDYTGFTISPIHATQVNNQTRTFISEKFGNQGDSLRFEQNNTTARYTLRGN
        ||:||||||:||||||||||||||||||||||||||||||||||||||:|||||||||||
        NGTMIHLAPEDYTGFTISPIHATQVNNQTRTFISEKFGNQGDSLRFEQSNTTARYTLRGN
      480       490       500       510       520       530

               550       560       570       580       590       600
        GNSYNLYLRVSSIGNSTIRVTINGRVYTATNVNTTTNNDGVNDNGARFSDINIGNVVASS
        ||||||||||||||||||||||||||||::|||||||||||||||||||||||||:|||:
        GNSYNLYLRVSSIGNSTIRVTINGRVYTVSNVNTTTNNDGVNDNGARFSDINIGNIVASD
      540       550       560       570       580       590

               610       620       630
        NSDVPLDINVTLNSGTQFDLMNIMLVPTNISPLY
        |::| ||||||||||| |||||||:||||: |||
        NTNVTLDINVTLNSGTPFDLMNIMFVPTNLPPLY
      600       610       620       630
```

Legend: Alignment of the amino acid sequences of Cry2Ab and Cry2Aa proteins.
| = identical AA; : = similar AA.

Figure 2. Cry2Ab Protein Sequence as Produced in Cotton. The sequence was deduced from the DNA used to transform corn. The chloroplast transit peptide is shown in italics (residues 1-79). The Cry2Ab protein corresponds to residues 80-713. The underlined amino acids (residues 77-79) correspond to the predicted portion of the chloroplast transit peptide remaining after processing. The amino acid at position 81 (D, aspartic acid) corresponds to the residue introduced for cloning purposes (position 2, Figure 1).

1 *MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG*

51 *LKKSGMTLIG SELRPLKVMS SVSTACMLA*M DNSVLNSGRT TICDAYNVAA

101 HDPFSFQHKS LDTVQKEWTE WKKNNHSLYL DPIVGTVASF LLKKVGSLVG

151 KRILSELRNL IFPSGSTNLM QDILRETEKF LNQRLNTDTL ARVNAELTGL

201 QANVEEFNRQ VDNFLNPNRN AVPLSITSSV NTMQQLFLNR LPQFQMQGYQ

251 LLLLPLFAQA ANLHLSFIRD VILNADEWGI SAATLRTYRD YLKNYTRDYS

301 NYCINTYQSA FKGLNTRLHD MLEFRTYMFL NVFEYVSIWS LFKYQSLLVS

351 SGANLYASGS GPQQTQSFTS QDWPFLYSLF QVNSNYVLNG FSGARLSNTF

401 PNIVGLPGST TTHALLAARV NYSGGISSGD IGASPFNQNF NCSTFLPPLL

451 TPFVRSWLDS GSDREGVATV TNWQTESFET TLGLRSGAFT ARGNSNYFPD

501 YFIRNISGVP LVVRNEDLRR PLHYNEIRNI ASPSGTPGGA RAYMVSVHNR

551 KNNIHAVHEN GSMIHLAPND YTGFTISPIH ATQVNNQTRT FISEKFGNQG

601 DSLRFEQNNT TARYTLRGNG NSYNLYLRVS SIGNSTIRVT INGRVYTATN

651 VNTTTNNDGV NDNGARFSDI NIGNVVASSN SDVPLDINVT LNSGTQFDLM

701 NIMLVPTNIS PLY

Figure 3. Cry2Ab Protein Sequence as Produced in Corn. The sequence was deduced from the DNA used to transform cotton. The chloroplast transit peptide is shown in italics (residues 1-79). The Cry2Ab protein corresponds to residues 80-713. The underlined amino acids (residues 77-79) correspond to the predicted portion of the chloroplast transit peptide remaining after processing. The amino acid at position 81 (D, aspartic acid) corresponds to the residue introduced for cloning purposes (position 2, Figure 1).

1 *MAPTVMMASS ATAVAPFQGL KSTASLPVAR RSSRSLGNVS NGGRIRCMQV*

51 *WPAYGNKKFE TLSYLPPLST GGRIRCMQA*M DNSVLNSGRT TICDAYNVAA

101 HDPFSFQHKS LDTVQKEWTE WKKNNHSLYL DPIVGTVASF LLKKVGSLVG

151 KRILSELRNL IFPSGSTNLM QDILRETEKF LNQRLNTDTL ARVNAELTGL

201 QANVEEFNRQ VDNFLNPNRN AVPLSITSSV NTMQQLFLNR LPQFQMQGYQ

251 LLLLPLFAQA ANLHLSFIRD VILNADEWGI SAATLRTYRD YLKNYTRDYS

301 NYCINTYQSA FKGLNTRLHD MLEFRTYMFL NVFEYVSIWS LFKYQSLLVS

351 SGANLYASGS GPQQTQSFTS QDWPFLYSLF QVNSNYVLNG FSGARLSNTF

401 PNIVGLPGST TTHALLAARV NYSGGISSGD IGASPFNQNF NCSTFLPPLL

451 TPFVRSWLDS GSDREGVATV TNWQTESFET TLGLRSGAFT ARGNSNYFPD

501 YFIRNISGVP LVVRNEDLRR PLHYNEIRNI ASPSGTPGGA RAYMVSVHNR

551 KNNIHAVHEN GSMIHLAPND YTGFTISPIH ATQVNNQTRT FISEKFGNQG

601 DSLRFEQNNT TARYTLRGNG NSYNLYLRVS SIGNSTIRVT INGRVYTATN

651 VNTTTNNDGV NDNGARFSDI NIGNVVASSN SDVPLDINVT LNSGTQFDLM

701 NIMLVPTNIS PLY

E. Human Health and Safety of the Cry2Ab Protein

The human health and safety of the Cry2Ab protein is based on (1) extensive animal toxicity testing of Cry proteins, including the highly homologous Cry2A class; (2) a history of safe consumption of Cry proteins by humans and; (3) results of *in vivo* and *in vitro* safety studies conducted with the Cry2Ab protein. These three results, described below, establish the absence of adverse effects in animals fed Cry proteins at exposures millions of times higher than estimated human dietary exposures.

1. Animal Safety Testing of Cry Proteins in *B.t.* Microbial Formulations

The low mammalian toxicity of *B.t.* microbial insecticide mixtures containing Cry proteins has been demonstrated in numerous safety studies (EPA, 1998b; Monsanto, 1997) conducted over the last 40 years. These include subchronic and chronic feeding studies and acute oral, dermal and inhalation studies in rats. Additionally, primary eye irritation, acute oral and dermal studies have been conducted in rabbits, and a subacute dietary study was conducted in humans. A number of these toxicology studies have been published (DeBarjac, *et al.*, 1980; Fisher & Rosner, 1959; Meeusen & Atallah, 1990; Shadduck, 1983; Siegel *et al.*, 1987). Extensive review of these studies by EPA, initially in 1982 and again in 1989, and the overall conclusion of lack of toxicity of Cry proteins led EPA to focus the testing requirements for microbial-derived products on acute oral, pulmonary and intravenous toxicity studies (EPA, 1989; Sjoblad *et al.*, 1992). Again in 1995, following review of all applicable safety studies conducted with *B.t.* products containing Cry proteins, EPA concluded that "Toxicology studies submitted to the U.S. Environmental Protection Agency to support the registration of *B. thuringiensis* subspecies have failed to show any significant adverse effects in body weight gain, clinical observations, or upon necropsy." EPA also concluded that "The large volume of submitted toxicology data allows the conclusion that the tested subspecies are not toxic or pathogenic to mammals including humans" (EPA, 1998b).

As a consequence of the low mammalian toxicity of *B.t.*, all of the microbial *B.t.* products approved since the first product approval in 1960 have been exempted from the requirement of a tolerance (EPA, 1998b). EPA has since established separate tolerance exemptions by amendment for various *B.t.* proteins expressed in transgenic plants, such as Cry1Ab, Cry1Ac, and Cry3Aa (EPA 1995a-d; 1996a,b; 1997).

Microbial *B.t.* products containing Cry2A proteins have also been tested and shown to be non-toxic when fed to animals (EPA, 1998b). Cry2A protein exhibits a high degree of amino acid similarity (97%) to Cry2Ab protein produced in cotton and corn (Figure 1). Thus, safety studies conducted with microbial *B.t.* products containing Cry2A proteins are relevant to the safety assessment of Cry2Ab protein. As shown in Table 1, Cry2A protein, as component of various *B.t.* microbial products, has been tested in acute, subchronic and chronic toxicity studies with rats, rabbits, sheep and humans. The highest doses administered to animals in these studies produced no observable effects (NOEL), consistent with the absence of toxicity of other Cry proteins when fed at high doses to animals.

Table 1. No Observed Effect Levels for Microbial *B.t.* Preparations Containing Cry2A Proteins

Test Substance [1]	Animal Model	NOEL [2]	Reference
		Acute Toxicity Studies	
Crymax	Rat	> 2.5-2.8 x 10^8 CFUs/rat	Carter & Liggett, 1994
Crymax	Rat	>5050 mg/kg	EPA, 1996b
Cutlass OF	Rat	> 10^8 CFUs/rat	David, 1989
Dipel	Rat	> 2670 mg/kg	EPA, 1996b
Dipel	Rat	> 3.4 x 10^{11} spores/kg.	EPA, 1986
Dipel	Rat	> 4.7 x 10^{11} CFUs/kg	EPA, 1986
Dipel	Rat	>5 000 mg/kg	EPA, 1986
Dipel	Rat	> 1.3 x 10^9 spores/kg	McClintock *et al.*, 1995
Dipel	Rabbit	>2x10^9 spores/animal	EPA, 1986
		Subchronic Toxicity Studies	
Dipel	Rat	8400 mg/kg/day/90 days	McClintock *et al.*, 1995
Dipel	Sheep	10^{12} spores/day/153 days	Hadley *et al.*, 1987
		Chronic Toxicity Study	
Dipel	Rat	8400 mg/kg/day/2 years	McClintock *et al.*, 1995
		Human Toxicity Study	
Dipel	Humans	1000 mg/day/5 days	McClintock, *et al.*, 1995; EPA, 1986

[1] Crymax contains Cry2A, Cry1Ac, Cry1C
Cutlass OF contains Cry2A, Cry1Aa, Cry1Ab, Cry1Ac, Cry2B
DIPEL contains Cry2A, Cry1Aa, Cry1Ab, Cry1Ac

[2] These NOELs represent the highest doses tested. Doses are expressed in various units for *B.t.* microbial technical grade materials *e.g.*, milligrams technical ingredient per kilogram body weight, or more commonly CFUs or spores per animal or kilogram body weight. It is not possible to directly compare doses on a milligram technical material per kilogram of body weight basis. This is due to the fact that colony-forming units (CFUs) or spore count can range from approximately 10^8 to 10^{11} per gram of technical grade *B.t.* microbial material (McClintock *et al.*, 1995). Secondly, the Cry protein content in different *B.t.* microbial preparations may vary depending on the microorganism and fermentation conditions. Cry2A protein dosages administered to animals in the referenced studies range from milligrams to grams per kilogram of body weight.

2. History of Safe Consumption of *B.t.* Residues on Food Crops

There is a history of safe dietary exposure to *B.t.* residues in or on raw agricultural commodities. EPA and WHO have recognized the potential for dietary exposure to Cry proteins from use of microbial sprays on food crops: "The use patterns for *Bacillus thuringiensis* may result in dietary exposure with possible residues of the bacterial spores on raw agricultural commodities. However, in the absence of any toxicological concerns, risk from the consumption of treated commodities is not expected for both the general population and infants and children" (EPA, 1998b) and "*B.t.* has not been reported to cause adverse health effects on human health when present in drinking-water or food." (IPCS, 1999).

B.t. microbial formulations have been applied for decades to raw agricultural commodities which are consumed in unprocessed form by humans. These include berry crops, cabbage, grapes, tomatoes, celery, lettuce, and spinach (EPA, 1998b). For certain crops, a significant percentage of the total amount grown in the United States has been treated with *B.t.* microbial preparations *e.g.*, raspberries (30%), celery (46%), and cabbage (39%) (EPA, 1998b). Residual levels of *B.t.* spores and microbes persist on foliage for several days following foliar application of microbial formulations (Leong, *et al.*, 1980; Dynamac, 1986). Thus, if commodities such as celery are consumed within a few days of application, there could be dietary exposure to *B.t.* microbes and spores, as well as to *B.t.* Cry proteins. There has been only limited sampling of raw agricultural commodities for *B.t.* residues; broccoli and cabbage leaves were reported to have mean residues of 10^6 to 10^7 *B.t kurstaki* (Dipel) spores/cm^2 leaf tissue (background *bacillus* counts on unsprayed leaves were < 10^2 spores/cm^2) (Leong *et al.*, 1980). In separate *B.t.* residue trials, residual *B.t. kurstaki* levels expressed as spores/gram plant tissue from days 0-7 post treatment were: 10^6 for celery (background not reported), 10^6 for collard greens (background 10^3), 10^6 for kale (background 10^3) and 10^6 for lettuce (background not reported) (Dynamac, 1986). Exposure has also been shown to natural populations of *Bacillus thuringiensis* strains that contain crystal proteins active against lepidoptera in granaries in Korea (Kim *et al.*, 1998); they are also ubiquitous in soils (IPCS, 1999). To the best of our knowledge, no measurement of residual Cry protein levels on raw agricultural commodities has been made following application of *B.t.* microbial sprays. Nevertheless, in consideration of the natural occurrence of endogenous *B.t.* organisms in soils and the widespread use of commercial microbial *B.t.* sprays for the last four decades, there is a history of safe dietary exposure to Cry proteins, including those of the Cry2A class, which are highly similar to the Cry2Ab protein that is the subject of this request.

3. Safety Testing of Cry2Ab Protein

Assessment of the human and animal safety of the Cry2Ab protein includes information characterizing the biological and physicochemical properties of the introduced protein, an assessment of the digestibility of the protein, and potential allergenicity and mammalian toxicity of the protein. Table 2 lists each of the protein safety assessment studies for Cry2Ab and the equivalent studies submitted previously for the Cry2Aa protein. The following sections summarize the results of numerous studies submitted by Monsanto as an amendment to Tolerance Petition PP 7F4888 (Docket Number PF-768) that demonstrate Cry2Ab is not toxic to mammals and presents acceptable risk to human safety. This Cry2Ab protein safety data is in agreement with similar data confirming the safety of the Cry2Aa protein, which is highly similar to the Cry2Ab protein.

Table 2. Summary of Protein Safety Studies for Cry2Ab and Cry2Aa.

Assessment Study	Test Substance	Results[1]
In vitro Digestive Fate	Cry2Ab	Half-Life <15 sec in SGF; Digested to stable tryptic core in SIF [2]
	Cry2Aa	Half-Life <15 sec in SGF; Digested to stable tryptic core in SIF [2]
Allergen Sequence Similarity	Cry2Ab	Not homologous to known protein allergens
	Cry2Aa	Not homologous to known protein allergens
Toxin Sequence Similarity	Cry2Ab	Not homologous to known protein toxins or other proteins of concern to human health
	Cry2Aa	Not homologous to known protein toxins or other proteins of concern to human health
Acute Mouse Oral Toxicity	Cry2Ab	No effects at highest dose tested, 1450 mg/kg
	Cry2Aa	No effects at highest dose tested, 4011 mg/kg

[1] Results for Cry2Ab can be found in Volumes 4-7 of the previously submitted tolerance exemption request for Cry2Ab (MRID Nos. 44966602-5). Results for Cry2Aa can be found in MRID Nos. 44235304-7; 44310303.

[2] SGF = Simulated Gastric Fluid; SIF = Simulated Intestinal Fluid

a) Characterization of the Introduced Cry2Ab Protein

Due to the extremely low levels of Cry2Ab protein produced in corn and cotton, it was necessary to produce sufficient quantities of Cry2Ab protein by bacterial fermentation for the development of analytical methods (*e.g.*, ELISA) and to conduct safety studies. Cry2Ab protein was produced in and purified from *Bacillus thuringiensis* strain EG7699. Characterization of this *B.t.* protein preparation was done using analytical methods and functional tests specifically selected to assess the identity, concentration, strength in bioassay, purity and composition. In addition, solubility and storage stability studies were performed. The Cry2Ab protein produced by *Bacillus thuringiensis* strain EG7699 was shown to have equivalent molecular weight and immunoreactivity to the protein expressed in cotton and corn, to lack detectable post-translational modification (glycosylation), to have equivalent electrophoretic mobility and detection with specific antibodies (Volume 6 of this submission), and to have similar functional activity. Thus, the Cry2Ab proteins derived from both bacterial fermentation and plant sources were established to be physicochemically and functionally equivalent.

b) Digestion of Cry2Ab Protein in Simulated Gastric and Intestinal Fluids
The purpose of this study was to assess the *in vitro* digestibility of Cry2Ab protein (apparent molecular weight of ≈63 kDa) using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) mammalian digestion models. The Cry2Ab protein was incubated in SGF and SIF at 37 °C for up to 2 hours and 24 hours, respectively. Protein stability was assessed at specific time points using SDS-PAGE (limit of detection, 10 ng; limit of resolution, ≥2 kDa) and/or immunoblotting (limit of detection, 5 ng; limit of resolution, ≥2 kDa) for each incubation. Moreover, a *Helicoverpa zea* insect bioassay (EC_{50}) was used to assess Cry2Ab protein functional activity remaining after selected incubation times.

SDS-PAGE analysis of SGF incubations showed that by 15 seconds greater than 98% of the Cry2Ab protein was digested and that no fragments ≥2 kDa of the parent protein were resolved. Immunoblot analysis of SIF incubations showed that a relatively stable Cry2Ab protein fragment (≈50 kDa) was produced within 1 minute and observed for at least 24 hours. Cry1, Cry2 and Cry3 class proteins yield stable, tryptic core fragments when incubated in SIF (Monsanto, 1997). These observations were corroborated by insect bioassays showing rapid loss of activity in SGF and stable activity in SIF.

This *in vitro* assessment of Cry2Ab protein digestibility indicates that the Cry2Ab protein will be readily digested in the mammalian stomach.

c) Allergenic Potential of the Cry2Ab Protein
Although large quantities of a range of proteins are consumed in human diets each day, rarely do any of these tens of thousands of proteins elicit an allergenic response (Taylor, 1992). Although there are no predictive bioassays available to assess the allergenic potential of proteins in humans (FDA, 1992), the physicochemical and human exposure profile of the protein provides a basis for assessing potential allergenicity by comparing it to known protein allergens. Thus, important considerations contributing to the allergenicity of proteins ingested orally includes exposure and an assessment of the factors that contribute to exposure, such as stability of digestion, prevalence in the food, and consumption pattern (amount) of the specific food (Metcalfe, *et al.*, 1996; Kimber *et al.*, 1999).

A key parameter contributing to the systemic allergenicity of certain food proteins appears to be stability to gastrointestinal digestion, especially stability to acid proteases like pepsin found in the stomach (Astwood *et al.*, 1996; Astwood and Fuchs, 1996; Fuchs and Astwood, 1996; FAO, 1995; Kimber *et al.*, 1999). Important protein allergens tend to be stable to peptic digestion and the acidic conditions of the stomach if they are to reach the intestinal mucosa where an immune response can be initiated.

Another significant factor contributing to the allergenicity of certain food proteins is their high concentrations in foods (Taylor, 1992; Taylor *et al.*, 1987; Taylor 1992, Fuchs and

Astwood, 1996). Most allergens are present as major protein components in the specific food representing from 2-3% up to 80% of total protein (Fuchs and Astwood, 1996). This is true for the allergens in milk (Taylor *et al.*, 1987; Baldo, 1984; Lebenthal, 1975; Taylor, 1986), soybeans (Shibasaki *et al.*, 1980; Burks *et al.*, 1988; Pederson and Djurtoft, 1989), and peanuts (Barnett *et al.*, 1983; Sachs *et al.*, 1981; Barnett and Howden, 1986; Kemp, 1985). In contrast to this generality for common allergenic proteins, Cry2Ab protein is present at low levels in these plants (<0.02%).

It is also important to establish that the protein does not represent a previously described allergen, and further, does not share potentially immunologically relevant epitopes (amino acid sequences recognized by IgEs). A database of protein sequences associated with allergy and coeliac disease was assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt) and from current literature. The keyword "allergen" was used to retrieve allergen sequences from the public domain databases. Additional unique allergens found in only current literature were appended, creating an updated database containing 567 unique protein sequences. The amino acid sequence of the Cry2Ab protein was compared to these sequences using the sequence alignment tool FASTA. Cry2Ab shares no structurally-significant sequence similarity to sequences within the allergen database and does not share potential immunologically-relevant amino acid sequences greater than seven contiguous identical amino acids. Cry2Aa also shares no significant sequence similarity with known allergen sequences (MRID 44966604).

d) Toxic Potential of the Cry2Ab Protein

1) Bioinformatics

The safety assessment of a protein expressed in genetically modified crops includes structural comparisons of the introduced protein with proteins associated with toxicity or other adverse health effects. Specifically, a biologically-relevant sequence similarity to a known toxin (*i.e.*, a sequence apparently derived from a common ancestor gene) may indicate that additional toxicological assessments be done.

A database of 4677 protein sequences associated with toxicity has been assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt). The amino acid sequence of the Cry2Ab protein was compared to sequences in this toxin database using the FASTA sequence alignment tool. In addition, the amino acid sequence of the Cry2Ab protein was compared to all protein sequences in publicly-available genetic databases to screen for structural similarity to pharmacologically-active proteins. Apart from expected similarities to other known crystal (Cry) proteins found in *Bacillus thuringiensis* and related species, no additional significant structural similarities were observed. The Cry family represents a diverse set of proteins derived from a common ancestor gene.

The results of these bioinformatics analyses indicates that the Cry2Ab protein is not similar to any toxin relevant to animal or human health. Likewise, the Cry2Aa protein shares no significant sequence similarity with protein toxins relevant to animal or human health (MRID 44966605).

2) Acute Oral Toxicity of Cry2Ab Protein in Mice

The low mammalian toxicity of *B.t.* microbial insecticide mixtures containing Cry2A protein has been demonstrated in numerous safety studies (Sjoblad, *et al.*, 1992). Acute administration is considered appropriate to confirm the safety of Cry2Ab, because proteins that are toxic typically act via acute mechanisms (Sjoblad *et al.*, 1992; Pariza and Foster, 1983; Jones and Maryanski, 1991).

Three groups of ten male and ten female mice were given acute, oral dosages of Cry2Ab protein at 67.3, 359 or 1450 mg/kg body weight, respectively. A separate group of ten male and ten female protein control animals received bovine serum albumin at a dose of 1200 mg/kg. The doses administered were designed to evaluate the potential hazards of the Cry2Ab protein at the highest acute oral dose that could be delivered to mice.

There were two unscheduled deaths, unrelated to the test substance, in the control groups. Since perforations of the esophagus were observed at necropsy, both deaths resulted from gavage injury. For all other mice (treated and control) there were no behavioral or other adverse clinical observations associated with test article administration. All mice gained weight throughout the study and food consumption was similar for all treated and control groups. The few gross necropsy findings at scheduled sacrifice are commonly observed in control mice. Since the necropsy findings present were distributed among the various treatment and/or control groups without any dose-response relationship and were low in frequency, none were considered test article-related.

There were no adverse effects attributed to the oral administration of Cry2Ab protein in male and female mice at doses of 67.3, 359, or 1450 mg/kg body weight. The No-Observed-Effect-Level (NOEL) for toxicity of Cry2Ab protein administered as an acute dose by gavage to mice was considered to be at least 1450 mg/kg, the highest tested dose. The highest dose administered represented the highest feasible dose based on test system capacity and protein solubility. This NOEL is comparable to those determined for other Cry proteins including the Cry2A class (Table 1).

3) Calculation of Exposure Margins for Consumption of Cry2Ab Protein in Food and Feed Derived from Insect-Protected Cotton and Corn.

In order to calculate exposure margins, it is necessary to first determine the expression levels of Cry2Ab in various corn and cotton tissues. Validated enzyme-linked immunosorbent assays (ELISAs) were used to estimate the Cry2Ab protein expression levels. For cotton, these samples included the seed, leaf, leaf collected over different timepoints during the season, whole plant and pollen. For corn, samples of young leaf, leaf collected over different timepoints during the season, pollen, silk, forage, grain and stover were analyzed. Samples were collected from numerous locations in the United States during 1998 field trials. These field sites provided a variety of environmental conditions representative of regions where cotton and corn are grown commercially.

i. Expression of Cry2Ab Protein in Insect-Protected Cotton

As described in Volume 3 of the tolerance exemption petition (MRID 44966601), accumulation of the protein in cotton was detected at low levels throughout the various plant tissues at various times throughout the growing season. The mean levels of Cry2Ab protein in young cotton leaf tissue ranged from 11.3 to 23.8 μg/g. In whole cotton plant tissues, the mean level of Cry2Ab protein ranged from 4.15 to 8.80 μg/g. The mean level of Cry2Ab protein in cottonseed tissue ranged from 37.1 to 43.2 μg/g, while in cotton pollen, the mean level of Cry2Ab protein ranged from <0.25 to 1.17 μg/g.

ii. Expression of Cry2Ab Protein in Insect-Protected Corn

As described in Volume 7 of this submission, production of the Cry2Ab protein in corn was highest in leaf (87 - 199 μg/g), followed by forage (16 - 30 μg/g), grain (1.5 - 2.6 μg/g), and lowest in pollen (0.15 - 0.25 μg/g). Expression of the Cry2Ab protein in silk and stover was determined to be 1 μg/g and 11 μg/g, respectively.

iii. Calculated Exposure Margins to Cry2Ab Protein from Human Consumption of Corn Grain and Cotton Seed Oil

There will be negligible human dietary exposure to the Cry2Ab protein present in transgenic cotton. The human consumable fractions of cotton are cottonseed oil and linters (National Cottonseed Products Assoc., 1990). Each is processed both chemically and thermally such that the *B.t.* protein likely would be removed or denatured (Sims *et al.*, 1996; Sims and Berberich, 1996).

Although exposure to Cry2Ab protein is considered to be negligible, a dietary exposure margin was calculated based on the worst case assumption that Cry2Ab protein could survive processing and be present at very low levels in cottonseed oil (Table 3). A dietary exposure margin for dairy cow consumption of cottonseed was also calculated.

The human dietary exposure margin for corn grain was calculated (Table 3) based on the worst case assumption that all Cry2Ab protein in corn grain survives processing. Dietary exposure margins for dairy cow consumption of corn grain and forage were also calculated.

In a two-year chronic rat feeding study with Dipel, a *B.t.* microbial formulation containing Cry2A protein, the NOEL was considered to be 8400 mg/kg/day (Table 2). Even if Cry2A protein represented only 1% of the product tested, the daily Cry2A dose over most of the rats' lifetimes would have been 84 mg/kg/day, which is also several of orders of magnitude higher than the worst case human exposures to Cry2Ab protein from consumption of corn- and cotton-derived food products (Table 3). Based on these extremely large exposure margins and absence of toxicity in animal safety studies, there would be no unreasonable risks to or adverse effects in humans or farm animals from consumption of food products derived from insect-protected corn and cotton.

Table 3. Calculated Dietary Exposure Margins

A. HUMANS

Food/Feed	Cry2Ab Level in Food/Feed	Daily Food/ Feed Consumed	Dose (mg/kg)	Exposure Margin[a]
Cottonseed oil	2.6×10^{-4} µg/gram[b]	0.07 grams/kg[c]	1.8×10^{-8}[d]	8×10^{10}
Corn grain	2.1 µg/gram	0.2 grams/kg[e]	4×10^{-4}	3.6×10^{6}

B. DAIRY COW

Food/Feed	Cry2Ab Level in Food/Feed	Daily Food/ Feed Consumed	Dose (mg/kg)	Exposure Margin[a]
Cottonseed	43.2 µg/gram	5.3 grams/kg[f]	2.3×10^{-1}	6.3×10^{3}
Corn grain	2.6 µg/gram	12.2 grams/kg[g]	3.2×10^{-2}	4.5×10^{4}
Corn forage	30.0 µg/gram	45.0 grams/kg[g]	1.35	1.1×10^{3}

[a] The exposure margin is calculated by dividing 1450 mg/kg, the NOEL in the Cry2Ab mouse gavage study, by the calculated mg/kg dose.

[b] Assumes that cottonseed protein is present in refined cottonseed oil at the limit of detection of the assay (1.3 µg protein/ml of oil) since none was detected. Cry2Ab represents 0.02% of the total protein in cottonseed (MRID # 44966601). Therefore, the level of Cry2Ab protein that might theoretically be present in cottonseed oil is 2.6×10^{-4} µg/ml. Assumes 1 ml = 1 gram.

[c] Total disappearance of cottonseed oil in food is estimated to be 900 X 10^{6} lbs/year (personal communication, National Cottonseed Products Association). Per capita US human consumption of cottonseed oil = 900 x 10^{6} lbs/US population (270 x 10^{6}) = 3.3 lbs/per capita/year, 4 grams/per capita/day, or 4 grams/60 kg = 0.07 grams/kg.

[d] The mg/kg dose would be 0.07 grams/kg x 2.6×10^{-4} µg/gram = 0.18×10^{-4} µg/kg = 1.8×10^{-8} mg/kg.

[e] USDA database (1990).

[f] Assumes a 600 kg dairy cow eats 7 lbs of cottonseed/day (Hoard's Dairyman, 1984). This is equivalent to 5.3 grams cottonseed/kg body weight/day.

[g] 7.3 kg grain, 27 kg forage consumed daily by dairy cows (NRC, 1989). 7.3kg grain /600 kg =12.2 grams/kg. 27 kg/ forage/600kg = 45 grams/kg.

4. Human Health and Safety Conclusions

The Cry2Ab protein has been shown to be safe for consumption by both humans and animals by the:

1. general recognition of the safety of *B.t.* proteins, including those of the Cry2A class;
2. high degree of sequence similarity of the encoded proteins of the *Cry2Ab* and the *cry2Aa* genes present in commercial *B.t.* formulations, which have a history of safe use;
3. rapid digestion of Cry2Ab in simulated gastric fluid (SGF) and conversion to the expected tryptic core protein in SIF, as expected for *B.t.* proteins;
4. lack of homology of Cry2Ab with known allergens ;
5. lack of homology of Cry2Ab with any known protein toxins or other proteins associated with adverse mammalian or human health effects;
6. lack of acute toxicity of Cry2Ab to mammals, as demonstrated by a mouse acute oral gavage study; and
7. low dietary exposure to the Cry2Ab protein from consumption of cotton and corn.

The toxicity and allergenicity profile for the Cry2Ab protein, as expressed in corn and cotton, indicates that there is an acceptable risk from exposure to the overall U.S. population. Furthermore, there has been no indication of mammalian toxicity from numerous *B.t.* proteins, regardless of their origin, degree of alteration, or level of expression. Therefore, there is a reasonable certainty that no harm will result from exposure of the U.S. population, including infants and children, to the Cry2Ab protein as produced in both cotton and corn.

F. Environmental Safety of Cry2Ab Protein

1. Environmental Safety Studies

To assess the environmental safety of Cry2Ab protein in insect-protected corn and cotton, 13 studies were conducted on bird, fish, aquatic invertebrate, and beneficial terrestrial invertebrate species (Vols. 8 - 20, this submission). Non-target organisms were exposed to high doses of leaf tissue, grain or pollen from insect-protected corn and/or cotton plants, or to Cry2Ab protein in diet for five days to eight weeks, depending on the study. The results discussed below, together with the history of safe use of *B.t.* proteins in general, demonstrate that Cry2Ab proteins in insect-protected corn and cotton pose no foreseeable risks to non-target organisms. No adverse effects were observed at the highest dose tested in all but a few studies (Table 4). In all cases the no observed effect concentration (NOEC) greatly exceeded the maximum environmental concentration indicating minimal risk.

Bobwhite quail and channel catfish fed insect-protected corn grain at 10% and 35% of their diets, respectively, and cottonseed at 10% and 20% of their diets, respectively, exhibited no mortality and no adverse effects on survival, growth or behavior. These data indicate that birds foraging on grain or seeds in insect-protected fields or fish fed grain or seeds as part of their diet, as in the aquaculture industry, will not be adversely affected. Moreover, a *Daphnia magna* study established the 48-hour EC_{50} to be greater than 120 mg Cry2Ab corn pollen/L, indicating that pollen containing a Cry2Ab protein concentration of 0.25 μg/g fresh weight is "practically non-toxic" to aquatic invertebrates according to hazard classification criteria in U.S. EPA, 1985, PB86-129269.

Studies were also conducted to determine whether non-target species of beetle and other terrestrial invertebrates are susceptible to Cry2Ab protein. Cry2Ab protein was evaluated in the ladybird beetle, as well as five other species of beneficial terrestrial invertebrates that could be exposed to Cry2Ab corn or cotton: adult and larval honey bees, Collembola, green lacewing, parasitic wasp, and earthworm. Cry2Ab poses minimal risk to these beneficial non-target organisms. No adverse effects were observed at the maximum predicted environmental concentration to which the organisms would be exposed. In most of the studies, the NOEC exceeded the maximum predicted environmental concentration by 10- to over 100- fold, demonstrating a wide margin of safety for these organisms (Table 4).

Cry2Ab is toxic to certain lepidopteran insects such as tobacco budworm or European corn borer. Studies conducted to determine the spectrum of activity of Cry2Ab against insect pests have established that Cry2Ab exhibits excellent activity in certain caterpillars (order Lepidoptera), but has no activity against other insect orders, including Coleoptera. Specifically, no effects on survival and/or growth were observed in studies with ladybird beetles and parasitic wasps at doses of 200 ppm in their diet.

2. Impact on Non-Target Organisms Including Endangered and Threatened Species

Considered in total, data provided in this submission and discussed above establish the safety of the Cry2Ab protein and *Bt* crops in general for beneficial and other non-target insects commonly found in corn and cotton fields. The absence of toxic effects in the non-target organism studies even at Cry2Ab levels considerably above the maximum predicted environmental exposure demonstrate that Cry2Ab will not have adverse impacts on these and related non-target organisms, including endangered and threatened species.

The potential of Cry1 and Cry2 proteins to effect non-target lepidopterans is well known, including the larvae of butterflies like the monarch, as well as endangered and threatened Lepidoptera. These Cry-expressing products pose no risk to endangered or threatened Lepidoptera, and negligible risk toward other non-target Lepidoptera, because such species will not be exposed to significant amounts of the proteins. None of these lepidopterans deliberately feed on cotton or corn plants, or tissues from such plants.

Table 4. Summary of Cry2Ab Protein Studies on Non-Target Organisms

Test Organism	Results	Test Substance	Conclusions
Bobwhite Quail	No mortality or toxic effects in birds consuming Cry2Ab corn grain or Cry2Ab cottonseed at 10% of diet	Cry2Ab corn grain Cry2Ab cottonseed	Cry2Ab corn grain or cottonseed poses minimal risk
Channel Catfish	No effect on growth or survival in fish consuming Cry2Ab corn grain or Cry2Ab cottonseed at 35% and 20% of diet, respectively	Cry2Ab corn grain Cry2Ab cottonseed	Cry2Ab corn grain and cottonseed can be used in catfish diet at up to 35% and 20%, respectively, with no adverse effects
Cladoceran (*Daphnia magna)*	EC_{50} > 120 mg Cry2Ab corn pollen/L	Cry2Ab corn pollen	Cry2Ab corn pollen is practically non-toxic to aquatic invertebrates
Adult Honey Bee	NOEC = 68 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 56X and 272X predicted maximum Cry2Ab concentration in cotton and corn pollen, respectively
Larval Honey Bee	NOEC = 170 µg Cry2Ab/ml , single dose	Cry2Ab protein	NOEC > 139X and 680X predicted maximum Cry2Ab concentration in cotton and corn pollen, respectively
Ladybird Beetle	NOEC = 4500 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 23X and 88X predicted maximum Cry2Ab concentration in corn and cotton leaf tissue, respectively
Collembola	NOEC = 69.5 µg Cry2Ab/g diet	Cry2Ab protein	NOEC > 18X and 17X maximum predicted environmental exposure to Cry2Ab protein from corn and cotton in soil, respectively
Green Lacewing Larvae	NOEC = 1100 µg Cry2Ab/g diet	Cry2Ab protein	NOEC > 6X and 22X maximum predicted environmental exposure to Cry2Ab protein from corn and cotton leaf tissue, respectively
Parasitic Hymenoptera (Wasp)	NOEC = 4500 µg Cry2Ab/ml diet	Cry2Ab protein	NOEC > 18,000X and 3700X maximum environmental concentration predicted in corn and cotton pollen, respectively
Earthworm	NOEC = 330 mg Cry2Ab/kg dry soil	Cry2Ab protein	NOEC ≥ 12X and 83X maximum estimated environmental exposure from corn and cotton in soil, respectively

Consequently, the only possible route of exposure to Cry2Ab for these species is through corn or cotton pollen drifting onto their host plant and being inadvertently consumed by the larvae . This requires that a species be sensitive to the Cry2Ab protein, be in the larval stages during the short 7-10 day period of pollen shed, and that the larval host plant be close enough to corn or cotton fields for pollen to be deposited on that plant. In addition, data provided in this submission demonstrate that the levels of Cry2Ab protein in corn or cotton pollen are very low and only substantial pollen deposition could cause any adverse effects to even an extremely sensitive species. Various studies of pollen dispersal have shown negligible wind dispersal of cotton pollen and very localized dispersal of corn pollen. In the latter case, pollen deposition declines rapidly (over 91%) even 1 meter outside a corn field and pollen is rarely found at all over 30 meters from a corn field (results of studies reported by G. Dively, J. Foster, R. Hellmich and M. Sears at the Monarch Butterfly Research Symposium in Chicago, IL on Nov. 2, 1999). Thus, significant exposure to Cry2Ab through corn pollen will only occur if the host plants are found within corn fields. Because the host plants of endangered and threatened Lepidoptera are not found in corn fields or in close association with corn fields, there will be no new risk of adverse effects to these species from Cry2Ab-containing pollen, compared with current practices. Indeed, common practices of herbicide use in corn fields will mean that, even for Lepidoptera that are not threatened, like the monarch butterfly that have abundantly distributed host plants, suitable host plants will only very rarely be found in corn fields and the risk of exposure to significant levels of Cry2Ab will be minimal.

Outcrossing to wild relatives is also not expected to result in effects on threatened or endangered animal or plant species either through direct toxic effects or competition. Outcrossing will not occur because cultivated cotton varieties and hybrid corn do not exist in the wild in the United States, nor are there wild relatives that can readily interbreed with corn or cotton in the areas of the United States where these crops are grown. Based on these observations, registration and commercialization of corn and cotton expressing the Cry2Ab protein will pose minimal risk to the environment with no predicted effects on threatened or endangered species.

3. Environmental Fate

Since plant expression of the Cry2Ab protein is under the control of a constitutive promoter, most parts of the cotton and corn plants express the gene and produce the protein throughout the growing season. Post-harvest plant residue from these improved crops may therefore contain small amounts of the insecticidally-active protein. At harvest, plant materials such as stalks, unharvested bolls or cobs, and leaf material are shredded and tilled into the soil to promote decomposition. EPA has conducted environmental assessments of other *B.t.* proteins and has issued findings of no significant impact (FONSI) for the Cry1Ac, Cry1Ab and Cry3A proteins (EPA, 1998b). *B.t.* protein crystals have been found to degrade readily in the field due to solar radiation and temperature (USDA, 1999b).

The environmental fate of highly similar purified *B.t.* proteins has been extensively studied. The published literature has demonstrated that *B.t.* protein adsorption to soil is rapid and complete within 30 minutes (Venkateswerla and Stotzky, 1992). Numerous other studies of the biodegradation and binding of *B.t.* proteins in soil have been conducted, including Tapp *et al.*, (1994), Tapp and Stotzky (1995; 1998), Crecchio and Stotzky (1997), Koskella and Stotzky (1997). These studies demonstrate that isolated *B.t.* proteins could bind to clay particles and humic acids in artificial soil mixes.

Significant increases in soil microbial populations, from 100- to 1000-fold, have been shown when protein degradation studies are conducted with transgenic plant tissue rather than purified protein (Donegan *et al.*, 1995). The EPA Corvallis laboratory has performed soil degradation studies with Bollgard cotton and NewLeaf®[3] potato plant tissues showing soil half-lives of between four and seven days (Palm *et al.*, 1993; 1994; 1996).

A soil degradation study conducted with the purified Cry2Aa protein, which is highly similar to Cry2Ab, determined the soil half-life based on biological activity to be 15.5 and 31.7 days for the laboratory and the field, respectively (MRID 44235308). These results demonstrate that the Cry2Aa protein, as a component of post-harvest plants, is expected to dissipate or degrade when cultivated into soil. Due to the high degree of similarity between the homologous Cry2Aa and Cry2Ab proteins, the Cry2Ab protein also is likely to dissipate or degrade under conventional cultivation in which cotton or corn residue is plowed into the soil after harvest.

4. Environmental Safety Conclusion

In summary, because of (1) the non-target organism studies conducted with the Cry2Ab protein summarized above; (2) the low likelihood of Cry2Ab exposure for threatened or endangered species; (3) the binding and biodegradation of *B.t.* proteins in the environment; and (4) the extensive history of safe use of *B.t.*, Monsanto Company concludes that registration and commercialization of corn and cotton producing the Cry2Ab protein will be safe to the environment and to non-target organisms.

G. Conclusions

Current agronomic practices in both corn and cotton continue to rely on extensive use of chemical insecticides to control insect pests, despite advances in IPM and insect-protected crops such as YieldGard corn and Bollgard cotton. Monsanto Company has developed new insect-protected cotton and corn products expressing the Cry2Ab protein that provide improved control of pests with a potential for reduced risk of resistance.

Registration under FIFRA requires EPA to determine that the Cry2Ab protein will not cause unreasonable adverse effects to human health and the environment under ordinary use conditions. We conclude, based on the weight of the evidence provided herein, that there is reasonable certainty that no harm to human health or the environment will result from the use of the Cry2Ab2 protein as produced in corn and cotton plants. We

[3] NewLeaf is a registered trademark of Monsanto Company.

respectfully conclude that the data and information in this and previous submissions fully support the EPA determinations necessary for registration of this protein under FIFRA.

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Section III.

REGISTRATION REQUEST

Proposed Labels for the Plant-Incorporated Protectant,
Bacillus thuringiensis Cry2Ab Insect Control Protein,
as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.)

NOTE: The subject of this submission is the registration of the plant-incorporated protectant, *Bacillus thuringiensis* Cry2Ab Insect Control Protein, as Produced in Corn (*Zea mays* L.) and Cotton (*Gossypium hirsutum* L.) for use in all corn and cotton lines and varieties. The proposed label is for the pesticidal protein as expressed within the plant cell.

Plant-Incorporated Protectant Active Ingredient
End Use Product

Cry2Ab Protein as Expressed in Corn Plants

Active Ingredient:

Bacillus thuringiensis Cry2Ab protein and the genetic material necessary for its production in corn..0.001 - 0.01%

Inert Ingredients: ....99.99 - 99.999%

* Percentage of whole plant on a dry weight basis

CAUTION
KEEP OUT OF THE REACH OF CHILDREN

Precautionary Statements

EPA REGISTRATION NUMBER 524 - LEE

EPA ESTABLISHMENT NUMBER 524-MO-002

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Directions for Use

It is a violation of Federal law to use this product in any manner inconsistent with its labeling.

Corn has been transformed to express the *Bacillus thuringiensis* Cry2Ab delta-endotoxin protein for the control or suppression of the following corn lepidopteran insect pests:

European Corn Borer	*Ostrinia nubilalis*
Southwestern Corn Borer	*Diatraea grandiosella*
Tobacco Budworm	*Heliothis virescens*
Corn Earworm	*Helicoverpa zea*
Cabbage Looper	*Trichoplusia ni*
Saltmarsh Caterpillar	*Estigmene acrea*
Cotton Leaf Perforator	*Bucculatrix thurberiella*
Soybean Looper	*Pseudoplusia includens*
Beet Armyworm	*Spodoptera exigua*
Fall Armyworm	*Spodoptera frugiperda*
Yellowstriped Armyworm	*Spodoptera ornithogolli*

Plant-Incorporated Protectant Active Ingredient
End Use Product

Cry2Ab Protein as Expressed in Cotton Plants

Active Ingredient:

Bacillus thuringiensis Cry2Ab protein and the genetic material necessary for its production in cotton.. 0.01 - 0.02% *

Inert Ingredients: .... 99.98 - 99.99% *

* Percentage of whole plant on a dry weight basis

CAUTION
KEEP OUT OF THE REACH OF CHILDREN

Precautionary Statements

EPA REGISTRATION NUMBER 524 -

EPA ESTABLISHMENT NUMBER 524-MO-002

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Directions for Use

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. Not for commercial planting in the following counties in the Texas panhandle, which historically are not cotton-producing counties: Dallam, Sherman, Hansford, Ochiltree, Lipscomb, Hartley, Moore, Hutchinson, Roberts, and Carson.

Cotton has been transformed to express the *Bacillus thuringiensis* Cry2Ab delta-endotoxin protein for the control or suppression of the following cotton lepidopteran insect pests:

Tobacco Budworm	*Heliothis virescens*
Pink Bollworm	*Pectinophora gossypiella*
Cotton Bollworm	*Helicoverpa zea*
Cabbage Looper	*Trichoplusia ni*
Saltmarsh Caterpillar	*Estigmene acrea*
Cotton Leaf Perforator	*Bucculatrix thurbeiella*
Soybean Looper	*Pseudoplusia includens*
Beet Armyworm	*Spodoptera exigua*
Fall Armyworm	*Spodoptera frugiperda*
Yellowstriped Armyworm	*Spodoptera ornithogolli*

Plant-Incorporated Protectant Active Ingredient
End Use Product

Bollgard II Cotton

Active Ingredients:

Bacillus thuringiensis Cry2Ab protein and the genetic material necessary for its production in cotton .... 0.01 - 0.02% *

Bacillus thuringiensis Cry1Ac protein and the genetic material necessary for its production in cotton .... 0.001 - 0.006%*

Inert Ingredients: .... 99.98 - 99.99% *

* Percentage of whole plant on a dry weight basis

CAUTION
KEEP OUT OF THE REACH OF CHILDREN

Precautionary Statements

EPA ESTABLISHMENT NUMBER 524-MO-002

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Directions for Use

It is a violation of Federal law to use this product in any manner inconsistent with its labeling. Not for commercial planting in the following counties in the Texas panhandle, which historically are not cotton-producing counties: Dallam, Sherman, Hansford, Ochiltree, Lipscomb, Hartley, Moore, Hutchinson, Roberts, and Carson.

Cotton has been transformed to express the *Bacillus thuringiensis* Cry2Ab delta-endotoxin protein for the control or suppression of the following cotton lepidopteran insect pests:

Tobacco Budworm	*Heliothis virescens*
Pink Bollworm	*Pectinophora gossypiella*
Cotton Bollworm	*Helicoverpa zea*
Cabbage Looper	*Trichoplusia ni*
Saltmarsh Caterpillar	*Estigmene acrea*
Cotton Leaf Perforator	*Bucculatrix thurbeiella*
Soybean Looper	*Pseudoplusia includens*
Beet Armyworm	*Spodoptera exigua*
Fall Armyworm	*Spodoptera frugiperda*
Yellowstriped Armyworm	*Spodoptera ornithogolli*

Section IV.

Plant-Incorporated Protectant Cry2Ab

DATA REFERENCE LIST

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 1

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
PRODUCT ANALYSIS:					
Characterization of Insect Protection Protein 2 (IPP2) Produced by Fermentation	44999301	9/30/99	99-782E	MONSANTO	OWN
Production of Tissue Samples from Insect Protected Cotton Events Grown in 1998 U.S. Field Trials		4/4/00	99-858E	MONSANTO	OWN
Production of Tissue Samples from Corn Events MON 840, MON 841 and MON 843 in the 1998 U.S. Field Trials		4/4/00	99-858E	MONSANTO	OWN
Amended Report for Molecular Characterization of Cotton Event 15985		4/4/00	99-858E	MONSANTO	OWN
Molecular Analysis of Corn Event MON 840		4/4/00	99-858E	MONSANTO	OWN
Protein Levels in Insect Protected Cotton Samples Produced in the 1998 U.S. Field Trials	44966601	11/5/99	99-782E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 2

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Insect Protection Protein 2 and NPTII Levels in Samples Collected from Corn Event MON 840 in the 1998 U.S. Field Trials		4/4/00	99-858E	MONSANTO	OWN
PRODUCT ANALYSIS:					
Assessment of the Equivalence of Proteins Expressed in Cotton Events 15813 and 15985	44939403	9/30/99	99-555E	MONSANTO	OWN
Assessment of the Equivalence of Proteins Expressed in Corn Event MON 840		4/4/00	99-858E	MONSANTO	OWN
TOXICOLOGY:					
Acute Oral Toxicity Study of Insect Protection Protein 2 in Mice	44966602	11/5/99	99-782E	MONSANTO	OWN
Bioinformatics Analysis of Insect Protection Protein 2 Sequence Utilizing an Allergen Database	44966604	11/5/99	99-782E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 3

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Bioinformatics Analysis of Insect Protection Protein 2 Sequence Utilizing Toxin and Public Domain Genetic Databases	44966605	11/5/99	99-782E	MONSANTO	OWN
Assessment of the *In vitro* Digestive Fate of Insect Protection Protein 2	44966603	11/5/99	99-782E	MONSANTO	OWN
Bacillus thuringiensis subsp. *kurstaki* P2A Insecticidal Protein (CryIIA Protein) Shares No Significant Sequence Similarity with Proteins Associated with Allergy or Coeliac Disease	44235304	2/28/97	96-185E	MONSANTO	OWN
Bacillus thuringiensis subsp. *kurstaki* P2A Insecticidal Protein (CryIIA Protein) Is Homologous to Proteins of the *Bacillus thuringiensis* Insecticidal Crystal Protein Gene Family, but not to Protein Toxins Found in Public Domain Sequence Databases	44235305	2/28/97	96-185E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 4

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Assessment of the Digestibility of Purified *B.t.k.* P2 Protein *in vitro* Using Mammalian Digestive Fate Models	44235306	2/28/97	96-185E	MONSANTO	OWN
Preparation and Confirmation of Doses for an Acute Oral Toxicity Study with *Bacillus thuringiensis* var. *kurstaki* Strain HD-1 (CryIIA) Protein in Albino Mice	44235307	2/28/97	96-185E	MONSANTO	OWN
Acute Oral Toxicity Study of *Bacillus thuringiensis* var. *kurstaki* strain HD-1 CryIIA (*B.t.k.* P2) Protein in Albino Mice	44310303	2/28/97	96-185E	MONSANTO	OWN
ENVIRONMENTAL FATE AND ECOLOGICAL EFFECTS:					
Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Honey Bee Larvae		4/4/00	99-858E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 5

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Evaluation of the Dietary Effect(s) of Insect Protection Protein 2 on Adult Honey Bees (*Apis melifera* L.)		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2: A Dietary Toxicity Study with Green Lacewing Larvae (*Chrysoperla carnea*)		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2: A Dietary Toxicity Study with Parasitic Hymenoptera (*Nasonia vitripennis*)		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2: A Dietary Toxicity Study with the Ladybird Beetle (*Hippodamia convergens*)		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2 in Corn Pollen: A 48-Hour Static-Renewal Acute Toxicity Test with the Cladoceran (*Daphnia magna*)		4/4/00	99-858E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 6

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Insect Protection Protein 2: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate		4/4/00	99-858E	MONSANTO	OWN
Assessment of Chronic Toxicity of Cotton Tissue Containing the Insect Protection Protein 2 to Collembola (*Folsomia candida*)		4/4/00	99-858E	MONSANTO	OWN
Assessment of Chronic Toxicity of Corn Tissue Containing the Insect Protection Protein 2 to Collembola (*Folsomia candida*)		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2 in Cottonseed Meal: A Dietary Toxicity Study with the Northern Bobwhite		4/4/00	99-858E	MONSANTO	OWN
Insect Protection Protein 2 in Corn Grain: A Dietary Toxicity Study with the Northern Bobwhite		4/4/00	99-858E	MONSANTO	OWN

Plant-Incorporated Protectant Cry2Ab
Data Reference List - Page 7

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Evaluation of Cottonseed Meal Derived From Insect Protected Cotton Lines 15813 and 15985 as a Feed Ingredient for Catfish		4/4/00	99-858E	MONSANTO	OWN
Evaluation of Insect Protected Corn Lines MON 840 and MON 841 as a Feed Ingredient for Catfish		4/4/00	99-858E	MONSANTO	OWN
Evaluation of the Dietary Effect(s) of Purified *Bacillus thuringiensis* subsp. *kurstaki* HD-1 P2 Protein on Honey Bee Larvae	44310304	2/28/97	96-185E	MONSANTO	OWN
Evaluation of the Dietary Effect(s) of Purified *Bacillus thuringiensis* subsp. *kurstaki* HD-1 P2 Protein on Adult Honey Bees	443410305	2/28/97	96-185E	MONSANTO	OWN
Bacillus thuringiensis subsp.*kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with Green Lacewing Larvae *(Chrysoperla carnea)*	44235313	2/28/97	96-185E	MONSANTO	OWN

	EPA REFERENCE NUMBER	SUBMISSION DATE	DOCUMENT NUMBER	OWNER	AUTHORIZATION
Bacillus thuringiensis subsp.*kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with Parasitic Hymenoptera *(Nasonia vitripennis)*	44235314	2/28/97	96-185E	MONSANTO	OWN
Bacillus thuringiensis subsp.*kurstaki* Strain HD-1 [CryIIA] Protein: A Dietary Toxicity Study with the Ladybird Beetle *(Hippodamia convergens)*	44235312	2/28/97	96-185E	MONSANTO	OWN
CryIIA Protein from *Bacillus thuringiensis* subsp.*kurstaki* (B.t.k. P2 Protein): An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate	44235316	2/28/97	96-185E	MONSANTO	OWN
Effect of the *Bacillus thuringiensis* insecticidal proteins CryIA(b), CryIA(c), CryIIA, and CryIIIA on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola)	44235315	2/28/97	96-185E	MONSANTO	OWN

Section V.

REGISTRATION REQUEST

PRODUCT ANALYSIS

The Data Reference List for the Plant-Incorporated Protectant, Cry2Ab, can be found in Section III of this volume. The specific studies detailing the molecular characterization of Cry2Ab in cotton and corn can be found in Volumes 4 and 5 of this submission, respectively. The study detailing the expression of the protein in cotton has been previously submitted, MRID 44966601 and the study detailing the expression of the protein in corn can be found in Volume 7 of this submission.

Section VI.

REGISTRATION REQUEST

RESIDUE DATA

Monsanto has requested an exemption from the requirement of a tolerance for the Plant-Incorporated Protectant, Cry2Ab, based on the mammalian safety (Petition PP 7F4888) and therefore requests that the requirements for residue data be waived for this product. Analytical methods for the detection and measurement of the Cry2Ab protein are therefore not necessary.

Section VII.

REGISTRATION REQUEST

NON-TARGET ORGANISM DATA

The Data Reference List for the Plant-Incorporated Protectant, Cry2Ab, can be found in Section III of this volume. Specific studies detailing the effects of the Cry2Ab protein on non-target organisms can be found in Volumes 8-20 of this submission. Additionally, specific studies detailing the non-target organism effects of the Cry2Aa protein, which is highly homologous to the Cry2Ab protein, have been previously submitted and are referenced in support of this registration request:

MRID numbers: 44310304, 44235310-17

Section VIII.

REGISTRATION REQUEST

TOXICOLOGY DATA

The Data Reference List for the Plant-Incorporated Protectant, Cry2Ab, can be found in Section III of this volume. The toxicology data for Cry2Ab has been previously submitted to support the request for exemption from the requirement of a tolerance (PP 7F4888). These referenced reports are contained in MRID numbers: 44966602-5.

Section IX.

REGISTRATION REQUEST

EFFICACY DATA

Monsanto Company requests the requirement to submit efficacy data be waived under provision 3(c)5 of FIFRA as amended.

Section X.

TOLERANCE REQUEST

A request for the exemption from the requirement of a tolerance has been previously submitted to the Agency (Petition PP 7F4888). The administrative materials in support of this request are contained in MRID 44966600 and the supporting data provided in MRIDs 44966601 - 44966605.